



obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR, preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identified by the method referred to above. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a method of producing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, under conditions suitable for obtaining expression of the hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof.

Brief Description of the figures

Figure 1 is an alignment of hVR1 *in silico* derived clusters with rat VR1. Figure 2 displays the human VR1 nucleotide sequence including the 5'UTR (nt – 773 to nt 0), coding region (nt 1 to 2517) and 3'UTR (nt 2518 to nt 3560) (SEQ ID NO:1). Figure 3 illustrates the nucleotide and encoded amino acid sequence of the human VR1 sequence (SEQ ID NO: 1 and SEQ ID NO:2). Figure 4 depicts the amino acid sequence (SEQ ID NO: 2) of the hVR1 gene, the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed). The predicted phosphorylation sites are underlined. Figure 5 is a comparison of the amino acid sequences of the rat (rVR1) (SEQ ID NO:3) and human (hVR1) vanilloid receptors (SEQ ID NO:2).

Figure 6 illustrates constructs pBluescriptSK(+) (A) and pCIN5-new (B) with the full length hVR1 gene cloned via NotI and EcoRI restriction sites.

Figure 7 shows a Slot Blot hybridisation with hVR1 probe with positive labelling of both rat and human DRG mRNA.

5 Figure 8 displays a Western blot probed with anti-VR1 antibodies with the arrow indicating the VR1 specific protein.

Figure 9 shows localisation of VR1 in rat DRG tissue sections, the arrow points to VR1 expressing small diameter (<25µm) neurone cell bodies.

10 Figure 10 depicts the *in situ* localisation of VR1 in human DRG sections (A) and human skin (B).

Figure 11 illustrates the functional response to capsaicin and blockade by capsazepine (CPZ) (A) with the current voltage relationship plotted in (B) on human VR-1 channels, transiently expressed in HEK293T cells.

15 Figure 12 shows capsaicin-induced desensitisation of human VR-1 channels in the presence of 2mM external calcium (A), maximum current (65mV) against time (B) and current voltage relationship in the absence of Ca^{2+} (C).

Figure 13 shows the influx of calcium into transiently transfected HEK293T cells over a time course in the presence of agonist capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

Figure 14 illustrates a graphical presentation the results shown in figure 13 examining the response of hVR1 transfected HEK293T cells over time before and after exposure to agonists: capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

25 Figure 15 displays the proposed assay strategy to carry out drug screening.

Figure 16 displays an alignment of *in silico* derived hVR3 specific clusters with rat VR1.

30 Figure 17 depicts the hVR3 nucleotide sequence including the 5' UTR (nt -686 to nt 0) Coding region (nt1 to nt 2889), 3'UTR (nt 2890 to nt 3418) (SEQ ID NO:4).

Figure 18 shows the nucleotide and amino acid sequence of hVR3 (SEQ ID NO:4 and SEQ ID NO:5).

35 Figure 19 is of the amino acid sequence of hVR3 (SEQ ID NO:5), the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

8 (cont.)

Figure 20 displays constructs pBluescriptSK(+) (A) and pCDNA3.1 (+) (B) with the full length hVR3 gene cloned via NotI and XhoI restriction sites.

Figure 21 illustrates a multiple comparison of the amino acid sequences of the rat VR1 (SEQ ID NO:3) and the human vanilloid receptors: hVR1, hVRL-1 and hVR3 (SEQ ID NO:2), (SEQ ID NO:6) and (SEQ ID NO:5), respectively.

5 Figure 22 Northern Blot hybridisation with hVR3 probe with strong signals detected in trachea (A), prostate (B), placenta, kidney and pancreas (C).

Detailed Description of the Invention

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising",
10 "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

As referred to above, the present invention relates to isolated human vanilloid
15 receptor (hVR) proteins, and in particular to the human vanilloid receptors which will be termed respectively human vanilloid receptors 1 and 3 (hVR1, and hVR3), sequence information for which is provided in figures 2 (hVR1) and 17 (hVR3). In the context of this invention the term "isolated" is intended to convey that the receptor protein is not in its native state, insofar as it has been purified at least to
20 some extent or has been synthetically produced, for example by recombinant methods. The term "isolated" therefore includes the possibility of the receptor protein being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, organic or inorganic solvents, or other materials where appropriate, but excludes the
25 situation where the receptor protein is in a state as found in nature.

Routine methods, as further explained in the subsequent experimental section, can be employed to purify and/or synthesise the receptor proteins according to the invention. Such methods are well understood by persons skilled in the art,
30 and include techniques such as those disclosed in Sambrook, J. *et al.* (28), the disclosure of which is included herein in its entirety by way of reference.

By the term "variant" what is meant throughout the specification and claims is that other peptides or proteins which retain the same essential character of the
35 human vanilloid receptor proteins for which sequence information is provided, are also intended to be included within the scope of the invention. For example,

The compounds may be administered via enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intraarterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

- 5 The present invention will be further explained, by way of examples, in the appended experimental section. Reference examples are provided.

Experimental details

10 **Reference Example A: Identification of related human ESTs (Expressed Sequence Tags) (19) to the rat VR1 sequence by *in silico* analysis**

The full-length rat VR1 amino acid sequence (15) was used as a query sequence using the tBlastn (20) alignment program to identify related human
15 genes in the dbEST (21) and Incyte (Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, California 94304, USA) databases. Several human ESTs were identified and those with similarities greater than 50% selected for further analysis. One of these ESTs was T12251 previously shown to have 68% amino-acid identity and 84% similarity over a region of 70 amino acids (15). Full-length
20 cloning and functional characterisation of the gene represented by this cluster has been completed (30). This gene was denoted hVRL-1 and encoded a protein of 764 amino acid protein (SEQ ID NO:6) that was 48 % identical to the rat VR1 protein. All human ESTs from both databases were clustered to identify overlapping identical ESTs belonging to the same transcript. The GCG package
25 (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin) and a program developed in house termed ESTBlast (22) were used to build up these clusters. In total, forty-three ESTs derived from different tissue sources and both EST databases were clustered into ten groups, one of these clusters represented hVRL-1. The remaining nine clusters have been named
30 hVRa, hVRb, hVRc, hVRd, hVRe, hVRf, hVRg, hVRh and hVRi. For each EST the tissue source was assigned according to the annotations in the dbEST and Incyte databases. Since no obvious starting codon was present and the cluster sequences were shorter than the rat VR1 transcript none of these clusters were likely to represent a full-length vanilloid receptor transcript. Finally hVRg, hVRh
35 and hVRi collapsed into a single contig. Sequence analysis has shown that

Reference Example B2: Sequencing of clones

All DNA sequences were determined by automated DNA sequencing based on the dideoxy chain-termination method using the ABI 373A / 377 sequencers (Applied Biosystems). Sequence-specific primers were used with the 'Big-Dye' Terminator Cycle Sequencing kit (Applied Biosystems). The nucleotide sequence was analysed using programs from the University of Wisconsin Genetics Computer Group package.

More specifically when sequencing an EST clone, the following protocol was adopted. The EST1 clone was grown using standard procedures and DNA was isolated using Qiagen columns. SP6 (5' ATTTAGGTGACACTATAG) (SEQ ID NO:7) and T7 (5' TAATACGACTCACTATAGGG) (SEQ ID NO:8) primers flanking the cloning site were used to sequence both ends. Plasmid DNA (0.6 pmol) was used with 10.0 pmol of each primer for the dye terminator reaction. The SP6 end corresponded to the *in silico* derived EST sequence (identical to EST1). The T7 end did not have homologies with VR1 nor did it possess a long open reading frame or a polyadenylation motif. The size of the insert was determined by enzyme digestion of the DNA with the endonucleases NotI and EcoRI and calculated to be approximately 3kb.

Plasmid DNA (50ng) was used to amplify the insert by Polymerase Chain Reaction (PCR) with T7 and SP6 as primers. The PCR conditions included an initial hot-start at 94°C for 2 minutes, followed by 35 cycles at 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 1 minute and terminated by 5 minutes at 72°C. The resulting PCR amplicon was separated on a 1.2% agarose gel and shown to be of ~3kb in size.

To fully sequence the PCR product the nuclease-Bal-31 technique was used where both strands of duplex DNA are degraded from both ends (23). After ethanol precipitation of the PCR product, the pellet was re-suspended in 30ml of 1X Bal-31 buffer (add buffer composition). A time-course digest with 2 units of Bal-31 enzyme (Roche Molecular Biochemicals) was carried out with 12 time points taken over 90 minutes (30 seconds, 1, 2, 3, 5, 7, 10, 15, 25, 45, 75 and 90 minutes). Three pools were made respectively from digests 1 to 4, 5 to 8 and 9

to 12. Each pool was blunt-ended and sub-cloned into the pCR-Script SK (+) plasmid from Stratagene at the SrfI site. After transformation, 16 colonies from each pool were screened by PCR with the flanking Reverse (5' GGAAACAGCTATGACCATG) (SEQ ID NO:9) and M13-20 (5' GTAAAACGACGGCCAGT) (SEQ ID NO:10) primers. The amplicons of 6 positive colonies per pool were subjected to direct sequencing (24) using the T3 (5' AATTAACCCTCACTAAAGGG) (SEQ ID NO:11) and T7 primers. The DNA sequences obtained were assembled using the GCG package, translated and aligned against the rat VR1 gene using the Blast tools. After analysis, the 3079bp amplicon was shown to have 2 introns of 603bp and 1221bp. The latter intron was located at the 3'end of the PCR product. The coding sequence covered 1255 bp and was separated by the former intron. Therefore the clone EST1 was likely to be a partially spliced and incomplete cDNA.

The clone belonging to cluster 1b (EST3) and derived from a kidney cDNA library was ordered and sequenced using the Bal-31 technique. After assembly of the sequences using the GCG package an identical overlap was identified with the DNA sequence of the cluster hVRc. Moreover a 3'end with a polyadenylation signal and tail was identified. The complete sequence of the combined hVRb Bal-31 derived sequence and hVRc was 2063 bp (1020 bp of coding and 1043 bp of 3' untranslated sequence).

Reference Example B3: Amplification of the middle section of hVR1 using the Polymerase Chain Reaction

We formulated the hypothesis that both sequences (hVRa and hVRb/c) were part of a common transcript. If the human and rat VR1 were going to be similar, the 2 contigs should be separated by a gap of approximately 275bp. Primers were designed on both sides of the gap to amplify mRNA from brain tissues in order to clone the gap. A smear was obtained with the sense primer (5' TCTACTTCGGTGAAGTGGCC) (SEQ ID NO:12) and antisense (5' ACGGCAGGGAGTCATTCTTC) (SEQ ID NO:13). For specificity 50ng of the PCR product were amplified with the nested sense (5' CTGCAGAACTCCTGGCAGA) (SEQ ID NO:14) and antisense (5' GTCACCACCGCTGTGGAAAA) (SEQ ID NO:15) primers. The 900bp nested amplicon was sequenced and shown to be identical to hVRa at one end and

hVRb/c at the other end. The middle part of the PCR product was homologous to the rat VR1 sequence. This region corresponded to 91 amino acids. When the sequences of hVRa, hVRb/hVRc and the internal amplicon are combined the total length of the Open Reading Frame (ORF) is 824 amino acids followed by a 3' untranslated sequence of 1043 bp. The human amino acid sequence is 87% identical to the rat sequence over that part of the coding region. This sequence was termed hVR1 because of its high degree of identity with the rat VR1 sequence.

Reference Example B4: Isolation of the 5' Terminus of hVR1 by PAC isolation

Since no start codon was identified at the 5' end an additional strategy was designed to identify the full-length sequence. Two primers, sense (5' TCCTCTGGCTTCCAACCCGTT) (SEQ ID NO:16) and antisense (5' GAACTGGGCAGAAAGTGCCT) (SEQ ID NO:17) were designed to amplify a 150bp product from the first intron mentioned in reference example B2. A P1 Artificial Chromosome (PAC) genomic clone (25) was isolated by PCR screening of a PAC library (Genome Systems, St Louis, Missouri). PAC DNA was recovered by using standard plasmid isolation protocol (26). An anti-sense primer was designed (5' CTGGAGTTAGGGTCTCCATCC) (SEQ ID NO:18) to sequence the genomic clone towards the potential 5' end of the gene. An open reading frame with a starting codon was identified. The gene structure was confirmed by using the GenScan software (27). The complete gene has a nucleotide sequence of 2517bp (figure 2) and encoded a 839 amino acid protein (Figures 3 and 4). The gene was named hVR1. Multiple alignment of the amino acid sequence of hVR1 and rat VR1 shows a remarkable degree of identity and similarities between both sequences (figure 5). The rVR1 and hVR1 amino acid sequences are 86% identical. Moreover after protein analysis 6 trans-membrane domains and 3 ankyrin binding domains were identified in hVR1 as in the rat VR1 gene.

Example 1: Full-length Amplification of hVR1 from human DRG and assembly into cloning vectors

HVR1 was PCR amplified in three sections from human DRG template. The 5' fragment was amplified using a sense primer encoding a NotI site and a strong

Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCCGCCACCATGAAGAAATGGAGCAGCAC) (SEQ ID NO:19) and an antisense primer (5' AGGCCCACTCGGTGAACTTC) (SEQ ID NO:20). The thermo-cycling conditions used for this amplification included a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min. A final extension step of 72°C for 5 min completed the reaction. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR®II-TOPO according to the manufacturers instructions supplied with the TOPO™ TA Cloning® kit (Invitrogen). The middle section of hVR1 was PCR amplified using the sense primer: 5' GACGAGCATGTACAATGAGA (SEQ ID NO:21) and antisense primer: 5' GTCACCACCGCTGTGGAAAA (SEQ ID NO:22). The cycling conditions included a hot start at 94°C for 4 mins, followed by 35 cycles of 1 min at 94°C, 56°C and 72°C. A final extension step of 72°C for 5 min completed the reaction. A band of approximately 870 bp was excised from a 2 % agarose gel and cloned as detailed by the TOPO™ TA Cloning® kit into the vector pCR2.1®-TOPO. Finally the 3' end was PCR amplified with the sense primer: 5' TGTGGACAGCTACAGTGAGA (SEQ ID NO:23) and the antisense primer: 5'TGCACTGAATTCGAGCACTGGTGTTCCTCAG (SEQ ID NO:24) which encoded an EcoRI site for cloning. The PCR conditions included a 90 sec hot start at 94°C followed by 35 cycles of 94°C for 50 sec, 50°C for 50 sec and 72°C for 50 sec. The cycling was completed with a 72°C step for 5 min. PCR products were separated on a 2% agarose gel and cloned into the vector pCR2.1®-TOPO.

Resulting clones for each of the three hVR1-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full length assembly of the gene. The NotI/DrallI (New England Biolabs) digested 5' end fragment ligated together with the middle DrallI/EcoRI fragment into a NotI/EcoRI restricted pBluescript SK (+) vector (Stratagene). Finally, the remaining 3' fragment was introduced into the resulting construct via MscI and EcoRI restriction sites, a map of the resulting construct is displayed in figure 6A.

Several clones were selected for sequence analysis to confirm that constructs still encoded the hVR1 consensus sequence. These were then digested with NotI/EcoRI and ligated into the mammalian expression vector pCIN5-new (a modified version of pCIN1 (32) having an IVS deletion as well as a 36 bp

deletion repositioning the start codon of neomycin phosphotransferase immediately after the upstream EMVC IRES) as illustrated in figure 6B.

Example 2: Chromosomal Localisation

The primers used to isolate the PAC clone (reference example B4) were selected for PCR on the G3 radiation hybrid panel from Stanford commercially available from Research Genetics (Huntsville, Alabama). The positive lanes and negative patterns were analysed using the public web server at Stanford University (<http://www-sghc.stanford.edu>). After analysis the hVR1 gene appears to be located on human chromosome 17 around marker SHGC-36073 (lod score=9.55).

Example 3: mRNA Distribution

The tissue distribution of hVR1 was established by slot-blot hybridisation. RNA was transferred onto a sheet of GeneScreen hybridisation transfer membrane (DUPONT) sandwiched in a slot blotter by suction via a vacuum pump. Once the membrane was rinsed in 2x SSC (3M sodium chloride and 0.3M sodium citrate pH7) for 2 min it was exposed to UV using an Ultraviolet crosslinker (Amersham Life Science) for 1min at 15000uW/cm² thus enabling cross-linkage of the RNA onto the membrane. The amounts of RNA on the blot are unknown. The probe was obtained by PCR amplification of a 260 bp product of the coding region of hVR1 with the following two primers: 5' TGTGGACAGCTACAGTGAGA (SEQ ID NO:25) and 5' GTGGAAAACCCGAACAAGA (SEQ ID NO:26). Membranes were hybridised for 4 hr shaking at 60°C in a 10% dextran sulphate, 1% SDS (sodium dodecyl sulphate) and 1M NaCl solution. The probe was labelled with [α 32P]dCTP (Amersham) using the Rediprime™DNA labelling system (Amersham), so as to obtain approximately 500,000cpm of the labelled probe per ml of prehybridisation solution. Briefly 100ng of probe was boiled for 3 minutes (denaturization) and then cooled on ice for 2 minutes in a total volume of 45µl. This was added to the labelling tube from the kit together with 3µl of 32P dCTP followed by an incubation at 37°C for 30 minutes. 400µl of Herring Sperm DNA (Sigma) at a concentration of 8µg/ml was added to the labelled probe and heated at 99°C for 3 minutes followed by rapid cooling on ice. The labelled probe was added and mixed well in pre-hybridisation solution. The membranes were hybridised overnight at 55°C.

The membranes were then washed, first at room temperature in 2xSSC and 1% SDS for 5 minutes, followed by 2x SSC and 1% SDS for 30 min at 50°C. If necessary further washes with 1x SSC and 0.5% SDS or 0.1xSSC and 0.1% for 30 mins at the same temperature were carried out. The membranes were then exposed to Scientific Imaging Film AR (Kodak) using intensifying screens at – 70°C overnight and the film developed.

The results are shown on figure 7. Strong signals were observed with the positive controls (slots 4B and 5B). Signals are detected on the human DRG slots (1A and 1B). No signals were detected with the water control (slot 3B). Three multi-tissue northern blots (Clontech) with a wide range of tissues have also been hybridised with the same probe, however no signals were detected. RT-PCR was performed on various tissues with the primer combination used to amplify the probe. A strong band was detected in DRG RNA. Taken together these hybridisations suggest that hVR1 is specifically expressed in neuronal tissue and DRG in particular.

Example 4: Design and production of Anti-hVR1 Antibody

The peptides CHFTTTRSRTLFGKGDSEEASC (SEQ ID NO:27) (peptide68) and CGSLKPEDAEVFKDSMVPGEK (SEQ ID NO:28) (peptide69) were synthesised by standard solid phase techniques and purified by gel filtration chromatography. These peptides were conjugated via their Cys residues to the carrier protein, Tuberculin PPD (purified protein derivative) using sulphy-SMCC (sulphosuccinimidyl 4-[N-maleimidomethyl]-cyclohexan-1-carboxylate). Rabbits, previously sensitised to Bacillus Calmette Guerin (BCG), were inoculated with the resulting conjugates emulsified in incomplete Freund's adjuvant at approx monthly intervals. Serum was prepared from blood samples taken 7 days after each immunisation. The specific antibody response was followed by indirect enzyme-linked immunosorbent assay (ELISA) using free peptide as antigen. Immunoglobulins were purified from high titre sera using immobilised peptide affinity columns (sulpholink Pierce). Rabbits designated M143, 144 and 145 received peptide68 conjugate, rabbits M146, 147 and 148, peptide69 conjugate.

The antibodies have been validated by specific staining of the recombinant protein expressed in HEK293 cells. Whole cell lysates were prepared in Sample

peak heights are reduced in cells pre-incubated in CPZ. The same FLIPR assay may be used to monitor the response of human VR1 on exposure to agonists and antagonists.

5 **Example 8: Example of a screen using human VR1.**

FLIPR assay technology may be utilised to screen for hVR1 modulators according to the procedure described in figure 15. Human VR1 may be gated with protons, capsaicin or heat.

10 **Reference Example C: Identification and partial characterisation of additional human vanilloid receptors (reference examples C1-C3):**

Reference Example C1: Identification and characterisation of a novel vanilloid-like receptor, hVR3

15 ESTs belonging to the remaining clusters were characterised by *in silico* cloning (reference example A). The following clones were used during this process: - EST6/EST7 (hVRd), -EST8. (hVRe), - EST9/EST10. (hVRf). These EST clusters have been aligned with rat VR1 in figure 16, note that this diagram is not to scale.

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Reference Example C2: Sequencing of clones

Further sequencing, as detailed in reference example B2, and *in silico* cloning, enabled clusters hVRd, hVRe and hVRf to collapse forming a single contig of 583 amino acids. This sequence was named hVR3 and has 49 % identity with the rat VR1 sequence. It was unlikely that this single contig was a full-length vanilloid receptor transcript as no obvious starting codon was present and it was shorter than the rat VR1 transcript.

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Reference Example C3: Identification of the 5' terminus of hVR3

30 Two primers (sense primer 5' ATGGCCACCAGCAGGGTTAC (SEQ ID NO:29) and antisense primer 5' TCTGCCAGGTTCCAGCTG) (SEQ ID NO:30) designed to PCR amplify an amplicon stretching the 3' end of hVR3 and its 3'utr were used to isolate a genomic PAC clone (Genome Systems. St Louis, Missouri). The hVR3 specific PAC clone was then used as template to generate a library.

35 This was achieved by sonicating 6µg of Qiagen purified PAC construct, size selecting fragmented DNA of 500-

2000bp. These resulting fragments were then blunt ended and cloned into the vector pCR®-Blunt as detailed in the manufacturers protocol supplied with the Zero Blunt™ PCR cloning kit (Invitrogen). Clones were then sequenced (reference example B2) to identify the complete 5' end of the hVR3 transcript. The full-length nucleotide sequence of the hVR3 gene is displayed in figure 17. Figure 18 illustrates both nucleotide and encoded amino acid sequence of the human VR1 and figure 19 depicts the amino acid sequence of the hVR3 gene with shaded regions denoting predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

Example 9: Full-length Amplification of hVR3 from human kidney template

Human kidney was used as a source of template for the PCR amplification of hVR3. Primers used for amplification were designed to isolate the gene in three fragments. Primers designed to isolate the 5' end included a sense primer encoding a NotI site and a strong Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCGCCACCATGCCCAGGGTAGTTGGAC (SEQ ID NO: 31) and antisense primer (5' CACCTCTTGTTGTCACTGGA) (SEQ ID NO:32). The PCR conditions used were a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min and finally one cycle at 72°C for 5 min. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR®II-TOPO according to the manufacturers instructions supplied with the TOPO™ TA Cloning® kit (Invitrogen). The middle fragment was PCR generated using sense and antisense primers 5' CAAATCTGCGCATGAAGTTCCAG (SEQ ID NO:33) and 5' GCCACGAGAAGTTCCACGTAGTG (SEQ ID NO:34) respectively in the presence of 5% DMSO. PCR thermo-cycling required 35 cycles of 1 min at 94°C, 58°C and 72°C for successful amplification of the fragment which was then excised from a 2% agarose gel for cloning into the pCR®II-TOPO vector. Finally the 3' fragment was amplified with a sense primer 5' GCTGCTCCCATCTTGCTGA (SEQ ID NO:35) and an antisense primer 5' TGCACTCTCGAGAAATGAGTGGGCAGAGAAGC (SEQ ID NO:36) encoding a XhoI restriction site. This fragment was successfully amplified using a hot start at 94°C for 4 min followed by 35 cycles of 94°C for 50 sec, 48°C for 50 sec and 72°C for 2 min. The cycling was completed with a 72°C step for 5 min. The amplified fragment was excised from a 2% agarose gel and clone into the pCR®II-TOPO vector.

Resulting clones for each of the three PCR generated hVR3-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full-length assembly of the gene. The DraIII restriction site of the pBluescript SK (+) vector (Stratagene) was firstly abolished by digestion with
 5 DraIII followed by a blunt ending step using T₄ DNA polymerase (New England Biolabs). This modified vector was then restricted to enable the ligation of both a NotI/NcoI 5' fragment and NcoI/ EcoRI middle fragment. Finally, the remaining 3' fragment was introduced into the resulting construct via DraIII and XhoI sites (figure 20A).

10 Several clones were selected for sequence analysis to confirm that the constructs still encoded the hVR3 consensus sequence. These were then digested with NotI/XhoI and ligated into the mammalian expression vector pCDNA3.1 (+) (Invitrogen) as seen in figure 20B. The resulting hVR3 consensus
 15 sequence is shown in the multiple alignment along with the full-length sequence of hVR1 and the published hVRL-1 in figure 21.

Example 10: Chromosomal localisation

20 The 3' terminus, including the 3' UTR sequence of hVR3 was used to design two primers to amplify a product of 360 bp: sense primer 5' ATGGCCACCAGCAGGGTTAC (SEQ ID NO:37) and antisense primer 5' TCTGCCAGGTTCCAGCTG (SEQ ID NO:38). The G3 radiation hybrid panel from Stanford University (Research Genetics, Huntsville, Alabama) was screened by PCR. The positive and negative lanes were analysed using the
 25 public web server at Stanford University (<http://www-sghc.stanford.edu>). After analysis the hVR3 gene appears to be located on human chromosome 12 around markers D12S177E (lod score=15) and D12S1893 (lod score=14).

Example 11: mRNA distribution

30 The following primers (5' ACAAGAAGGCGGACATGCGG (SEQ ID NO:39) and 5' ATCTCGTGGCGGTTCTCAAT) (SEQ ID NO:40) were used to obtain a PCR product from the coding region of hVR3. This amplicon was used as a probe on multi-tissue northern blots, the protocol of which is detailed in example 3, to determine the tissue distribution of the gene (figures 22A, 22B and 22C). A
 35 transcript of approximately 3.8 kb was detected in the following tissues (the intensities of the

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FIG. 2

hVR1 SEQUENCE INCLUDING THE 5'UTR (nt -773 TO nt 0), CODING
REGION (nt 1 TO 2517) AND 3'UTR (nt 2518 TO nt 3560) (SEQ ID NO:1)

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-773  cccccagccacacacacacacgcacacacatacacacacacacacaggttaaccattca  -714
-713  aaggccagaagcttgacagatgttgattcataaaatgcaaaagccaaaatccaaaatct  -654
-653  tgtataagctcagtggtgtggcagcgaggttgaagagcaaaggcaggccggggcacctgg  -594
-593  ctgatgatgtgtggaccggttgacagcagggcccgagtgcggtgtgggtgtgggtggg  -534
-533  ccagtctctgcccgtcacccatttccagggaacacagtctgcttggctcttctggactgag  -474
-473  ccatactcatcacccagatcctccctgaattcagcccacgacagccaccccgccgctttt  -414
-413  ccttggtctgtgtgggaagggaggcagcgcggtggttatcaacctcaccctgcagaggag  -354
-353  gcacctgagggccagagacgaggaggatgggtctaaccacagaaccacagatggctctga  -294
-293  gccgggggacctgtccacctcccaggccgacgtcagtgccgcaggactgcctggggcct  -234
-233  gctaggacctgctcacctctgaggacctctggggtgagaggttcagtcctggaaacacttca  -174
-173  gttctagggggctgggggcagcagcaagttggagttttggggtacctgttcacagggc  -114
-113  ccttggcaaggagggcaggtgggggtctaaggacaagcagtccttactttgggagtcaacc  -54
-53  ccggcgtggtggctgctgcaggttgcacactggggccacagaggatccagcaaggATGAAG  6
7  AAATGCAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA  66
67  GACCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG  126
127  GCCAAGAGCCGCACCCGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGAT  186
187  TGCCCTCACGAGGAAGGTGAGCTGGAAGTCTGCCCCGACCATCACAGTCAGCCCTGTTATC  246
247  ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTC  306
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FIG. 3

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR1 INCLUDING (SEQ ID NO:1)
THE 5'UTR (nt -773 TO nt 0), CODING REGION (nt TO 2517) AND
3'UTR (nt 2518 TO nt 3560) (SEQ ID NO:2)

-773	ccccccagccacacacacacacgcacacacacacacacacacagcgttaaccattca	-714
-713	aaggccagaagccttgacagatgttgattcataaaaatgcaaaagccaaaatccaaaatct	-654
-653	tgtataagctcagtggtgtggcagcgaggtgaagagcaaaggcaggccgggcacctgg	-594
-593	ctgatgatgtgtggaccgttgacagcagggcccgagtcggtgtgggtgtgggtggg	-534
-533	ccagtctctgcccgtcaccctattccagggacacagtcctgttggctcttcttggaactgag	-474
-473	ccatcctcatcacccgagatcctccctgaattcagcccacgacagccaccccgccgtttt	-414
-413	ccttggtctgtgtgggaagggaggcagcgcggtgttatcaacctcacctgcagaggag	-354
-353	gcacctgagggccagagacgaggagggatgggtotaaccagaaccacagatggctctga	-294
-293	gccggggggcctgtccaccctcccaggccgagtcagtgccgcaggactgcctggggccct	-234
-233	gctaggcctgctcacctctgaggcctctgggggtgagaggttcagtcctggaacacttca	-174
-173	gttctagggggctgggggcagcagcaagttggagttttggggtaccctgcttcacagggc	-114
-113	ccttggcaaggagggcaggtgggggtctaaggacaagcagtccttactttgggagtcacc	-54
-53	ccggcgtggtggctgctgcaggttgcacactggggccacagaggatccagcaaggATGAAG	6
1		M K 2
7	AAATGGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA	66
3	K W S S T D L G A A A D P L Q K D T C P	22
67	GACCCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG	126
23	D P L D G D P N S R P P P A K P Q L S T	42
127	GCCAAGAGCCGACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGAT	186
43	A K S R T R L F G K G D S E E A F P V D	62
187	TGCCCTCACGAGGAAGGTGAGCTGGACTCCTGCCCGACCATCACAGTCAGCCCTGTTATC	246
63	C P H E E G E L D S C P T I T V S P V I	82
247	ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTC	306
83	T I Q R P G D G P T G A R L L S Q D S V	102
307	GCCGCCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCCGTT	366
103	A A S T E K T L R L Y D R R S I F E A V	122
367	GCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAG	426
123	A Q N N C Q D L E S L L L F L Q K S K K	142
427	CACCTCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC	486
143	H L T D N E F K D P E T G K T C L L K A	162
487	ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGATCGCGCGG	546

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FIG. 4

AMINO ACID SEQUENCE OF hVR1 (SEQ ID NO:2)

1 MKKWSSTD LG AAADPLQKDT CPDPLDGD PN SRPPPAKPQL STAKSRTRLF
 51 GKG DSEEA FP VDCPHEEGEL DSCPTITVSP VITIQRPGDG PTGARLLSQD
 101 SVAASTEKTL RLYDRRSIFE AVAQNNCQDL ESLLLFLQKS KKHLTDNEFK
 151 DPETGKTCLL KAMLNLDHGQ NTTIPLLEI ARQDLSLDEL VNASYTDSYY
 201 KGQTALHIAI ERRNMALVTL LVENGADVQA AAHGDFFKKT KGRPGFYFGE
 251 LPLSLAACTN QLGIVKFLQ NSWQTADISA RDSVGN TVLH ALVEVADNTA
 301 DNTKFVTS MY NEILILGAKL HPTLKLEELT NKGMTPLAL AAGTGKIGVL
 351 AYILQREIQE PECRHLSRKF TEWAYGPVHS SLYDLSCIDT CEKNSVLEVI
 401 AYSSSETPNR HDMLLVEPLN RLLQDKWDRF VKR HFFENELVYCLYMIET
 451 MAAYYRPVDG LPPFKMEKIG DYFRVTGEIL SVLGGVYFFFA RGIQYFLQRR
 501 PSMKTLFV SYSEMTEFFLOS LPLMATVVLY PSHLKEYVAS MVESLALGWT
 551 NMDYYTRGEQ QMGIYAVMIE KMILRL LCRF MEVYIVFLFG FSTAVVILIE
 601 DGKNDSLPSE STSHRWGPA CRPPDSSYNS LYSTCLELFK FTIGMGDLEF
 651 TENYD EKAVF LILGLAYVIL ATYILHNMIL ALMGEETVNKI AQESKNIWKL
 701 QRAITILDTE KSFLKCMRKA FRSGKLLQVG YTPDGKDDYR WCFRVDEVNW
 751 TTWNTNVGII NEDPGNCXGV KRTLSFSLRS SRVSGRHWKN FALVPLLREA
 801 SARDRQSAQP EEVYLRQFSG SLKPEDAEVF KSPAASGEK*

Key

T/S predicted phosphorylation sites

 Transmembrane domains Ankyrin binding domains

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FIG. 5

COMPARISON OF THE AMINO ACID SEQUENCE OF THE RAT (VR1) (SEQ ID NO:3)
AND HUMAN (hVR1) VANILLOID PROTEINS. (SEQ ID NO:2)

	10	20	30	40	50
VR1	MEQRASLDSEESPPQENSCLDPPDRDPNCKPPPVKPHIETTRSRTRLF				
hVR1	MKKWSSTDLGAAADPLQKDTCPDPLDGDPNRPPPAKPQLSTAKSRTRLF				
	60	70	80	90	100
VR1	GKGDSEEA SPLDCPYEEGGLASCPITVSSVLTIORPGDGPASVRPSSQD				
hVR1	GKGDSEEA FVDCPHEEGELDSCPTITVSPVITIQRPGDGPTGARLLSQD				
	110	120	130	140	150
VR1	SVSAG . EKPPRLYDRRSIFDAVAOSNCQELESLLPFLORSKRRLTDSFK				
hVR1	SVAASTEKTLRLYDRRSIFEAVAQNNQDLESLLLELQSKKHLTDNEFK				
	160	170	180	190	200
VR1	DPETGKTCLEKAMNLNHNQNDTIALLLDVARKTDSLKQEVNASYTDSEY				
hVR1	DPETGKTCLEKAMNLNHDGQNTTIPLLLEIARQTDSEKELVNASYTDSEY				
	210	220	230	240	250
VR1	KGOTALHIATERRNMTLVTLVENGADVQAAANGDFFKRTKGRPGFYFGE				
hVR1	KGOTALHIATERRNMLVTLVENGADVQAAAHGDFFKRTKGRPGFYFGE				
	260	270	280	290	300
VR1	LPLSLAACTNQLAIVKFLQNSWQPADISARDSVGNITVLHALVEVADNTV				
hVR1	LPLSLAACTNQLGIVKFLQNSWQTADISARDSVGNITVLHALVEVADNTV				
	310	320	330	340	350
VR1	DNTKFVTSMYNEILILGAKLHPTLKLEETNRKGLTPLALAASSGKIGVI				
hVR1	DNTKFVTSMYNEILILGAKLHPTLKLEELTNKGMTPLALAAAGTGKIGVI				
	360	370	380	390	400
VR1	AYILQREIHEPECRHLSRKETEWAYGVPVHSSLYDLSCIDTCEKNSVLEVT				
hVR1	AYILQREIQEPECRHLSRKETEWAYGVPVHSSLYDLSCIDTCEKNSVLEVT				
	410	420	430	440	450
VR1	AYSSSETPNRHDMLLVEPLNRLLODKWDREVKRIEYFNFEVYCLYMIIFT				
hVR1	AYSSSETPNRHDMLLVEPLNRLLODKWDREVKRIEYFNFLVYCLYMIIFT				
	460	470	480	490	500
VR1	AAAYYRPVEGLPPYKLRWTVGDYFRVTGEILSVSGGVYFFERGIOYFLOR				
hVR1	MAAYYRPVDGLPPFKMEK . IGDYFRVTGEILSVLGGVYFFERGIOYFLOR				
	510	520	530	540	550
VR1	RPSLKSLEVDSYSEILFEVOSLFMLVSVVLYFSORKEYVASMVFSLAGW				
hVR1	RPSMKTLEVDSYSEMLFFELQSLFMLATVVLYFSLHKEYVASMVFSLAGW				
	560	570	580	590	600
VR1	TNMLYYTRGFOOMGIYAVMIEKMILRDLCRMVYLVFLEGFSTAVVTLLI				
hVR1	TNMLYYTRGFOOMGIYAVMIEKMILRDLCRMVYIVFLEGFSTAVVTLLI				
	610	620	630	640	650
VR1	EDGKNNSLPEMESTPHKCRGSACK . PGNSYNSLYSTCLELEFKETIGMGDLE				
hVR1	EDGKNDSLPSESTSHRWGRGPACRPDSSYNSLYSTCLELEFKETIGMGDLE				
	660	670	680	690	700
VR1	FTENYDEKAVFILLAYVILTYILLINMLIALMGETVNKIAOESKNITWK				
hVR1	FTENYDEKAVFILLAYVELTYILLINMLIALMGETVNKIAOESKNITWK				
	710	720	730	740	750
VR1	LQRAITILDTEKSELRKMRKAFRSGKLLQVGETPDGKDDYRWCFRVDEVN				
hVR1	LQRAITILDTEKSELRKMRKAFRSGKLLQVGYTPDGKDDYRWCFRVDEVN				
	760	770	780	790	800
VR1	WITWNTNVGLINEDPGNCEGVKRTLSFSLRSSGRVSGRNWKNFALVPLLRD				
hVR1	WITWNTNVGLINEDPGNCEGVKRTLSFSLRSSVSGRHWKNFALVPLLRE				
	810	820	830		
VR1	ASTDRRHATQEEVOLKHYTGSLKPEDAEEVFKDSMUPGK				
hVR1	ASARDRQSAQPEEVYTRQFSGSLKPEDAEEVFKSPAASGK				

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FIG. 17

hVR3 SEQUENCE INCLUDING 5' UTR (nt -686 TO nt 0) CODING
REGION (nt1 TO nt 2889), 3'UTR (nt 2890 TO nt 3418) (SEQ ID NO:4)

```
-684  ttacgcggttaagaaatacccaagcttatgcatcaagcttggtaccgagctcggatccact -625
-624  agtaccgccggccagtgtgctggaattcaagggtgaggagaggagcatggatcctggggagc -565
-564  gagtgtgtgcaggccaggaggggctttccagaggagcccagttgagctggaacaccagtg -505
-504  gggaggagttgaccagcaaagggtgcaggaggaggatcagcactttgcactggggagcagag -445
-444  tttgtgcactggggaagtcaactcaagtattggagcctcagtttctgttctgtaaaatg -385
-384  ggttcatcatgacagtgtttgatgaggaaaaggactgccggcctacacagcaagtccaca -325
-324  tggattttctgagccctcctgtgcctgaagccacggttaatggttctgccttagcagg -265
-264  tgcttaccacgtgcccaggcactgcactgcactggccactggactgcatgttctgtccatg -205
-204  aggcttggatatcccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct -145
-144  caatgtcatggaatgactaagtgtggagcctggatttgaacttggtctctctggggctcca -85
-84  aagctggctttcttggtcagcagtaggggtctgggatccaagtatgggggtcccagcttgac -25
-24  cctgaagtccaccctctttcagctaATGCCCAGGGTAGTTGGACCTGGGGCCAATTTGTG 35
36  TTTCCAGGTTCGTGAAAGAGGCTCCTGTTGCAGTTCCTCGCCTGAGGCTGGCGGCAACCA 95
96  CATCTGGGAGTGGCTCCCTGTGCCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC 155
156  AGCACTGCTGCTTGCTCCACGTGGGAGGGGGCTTCTGGAGCCCCCGCCCCCTGGCCGGGTT 215
216  CTGCCTGACTCCCCTTTCATTCCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG 275
276  CATGGCGGATTCCAGCGAAGCCCCCGCGCGGGCCCCGGGAGGTGGCTGAGCTCCCCGG 335
336  GGATGAGAGTGGCACCCCAGGTGGGGAGGCTTTTCCTCTCTCCTCCCTGGCCAATCTGTT 395
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FIG. 18

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR3 (SEQ ID NO:4)
INCLUDING THE 5'UTR (nt -684 TO nt 0), CODING REGION (nt1
TO 2889) AND 3'UTR (nt 2890 TO nt 3418) (SEQ ID NO:5)

-684	ttacgcgttaagaaataaccaagcttatgcatcaagcttggtaccgagctcggatccact	-625
-624	agtaccgcccggccagtggtgctggaattcaagggtgaggagaggagcatggatcctgggagc	-565
-564	gagtgtgtgcaggccagggagggcctttccagaggagcccagttgagctggaacaccagtg	-505
-504	gggaggagttgaccagcaaagggtgcaggggagggatcagcactttgcactggggagcagag	-445
-444	tttgtgcactggggaagtcaactcaagtattggagcctcagtttctctgttctgtaaaatg	-385
-384	ggttcatcatgacagtggttggatgagggaaaaggactgcggcctacacagcaagtccaca	-325
-324	tggattttctgagccccctcctgtgcctgaagcccacgggttaatggttctgccttagcagg	-265
-264	tgcttaccacgtgccaggcactgcactgcactggccactggactgcatgttctgtccatg	-205
-204	aggcttgatatccccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct	-145
-144	caatgtcatggaatgactaagtgtggagcctggatttgaacttggtctctctggggctcca	-85
-84	aagctggcctttcttggtcagcagtagggctctgggatccaagtatggggctccagcttgac	-25
-24	cctgaagtccaccctctttcagctaATGCCCAGGGTAGTTGGACCTGGGGCCAATTGTG	35
1	M P R V V G P G A N L C	12
36	TTTCCAGGTTTCGTGAAAGAGGCTCCTGTTGCAGTTCCCGCCTGAGGCTGGCGGCCAACCA	95
13	F Q V R E R G S C C S S R L R L A A N H	32
96	CATCTGGGAGTGGCCTCCCTGTGCCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC	155
33	I W E W P P C A P V I T T V A L K Q L A	52
156	AGCACTGCTGCTTGTCCACGTGGGAGGGGGCTTCCTGGAGCCCCCGCCCCCTGGCCGGGTT	215
53	A L L L V H V G G G F L E P P P L A G F	72
216	CTGCCTGACTCCCCTTTTCATTCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG	275
73	C L T P L S F P C R L S S A D G P G A G	92
276	CATGGCGGATTCCAGCGAAGGCCCCCGCGGGGGCCCGGGGAGGTGGCTGAGCTCCCCGG	335
93	M A D S S E G P R A G P G E V A E L P G	112
336	GGATGAGAGTGGCACCCAGGTGGGGAGGCTTTTCCTCTCTCCTCCCTGGCCAATCTGTT	395
113	D E S G T P G G E A F P L S S L A N L F	132
396	TGAGGGGGAGGATGGCTCCCTTTTCGCCCTCACCGGCTGATGCCAGTCGCCCTGCTGGCCC	455
133	E G E D G S L S P S P A D A S R P A G P	152
456	AGGCGATGGGCGACCAATCTGCGCATGAAGTTCCAGGGCGCCTTCCGCAAGGGGGTGCC	515
153	G D G R P N L R M K F Q G A F R K G V P	172
516	CAACCCCATCGATCTGCTGGAGTCCACCCTATATGAGTCCTCGGTGGTGCCTGGGCCCAA	575
173	N P I D L L E S T L Y E S S V V P G P K	192

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FIG. 19

AMINO ACID SEQUENCE OF hVR3 (SEQ ID NO:5)


```

1  MPRVVGPGAN LCFQVRERGS CCSSRLRLAA NHIWEWPPCA PVITTVALKQ
51  LAALLLVHVG GGFLEPPPLA GFCLTPLSFP CRLSSADGPG AGMADSSEGP
101 RAGPGEVAEL PGDESGTPGG EAFPLSSLAN LFEGEDGSLS PSPADASRPA
151 GPGDGRPNLR MKFQGAFRKG VPNPIDLLES TLYESSVVPK PKKAPMDSLK
201 DYGTyrHHSS DNKRWRKKII EKQPQSPKAP APQPPPIKLV FNRPILEDIV
251 SRGSTADLDG LLPFLLTHKK RLTDEEFREP STGKTCLPKA LLNLSNGRND
301 TIPVLLDIAE RTGNMREFIN SPFRDIYYRG QTALHIAIER RCKHYVELLV
351 AQGADVHAQA RGRFFQPKDE GGYFYEGELP LSLAACTNQP HIVNYLTENP
401 HKKADMRRQD SRGNTVLHAL VAIADNTREN TKFVTMYDL LLLKCARLFP
451 DSNLEAVLNN DGLSPLMAA KTCKIGIFQH IIRREVTDED TRHLSRKSKE
501 WAYGPFVYSSL YDLSSLDTCG EEASVLEILV YNSKIENRHE MLAVEPINEL
551 LRDKWRKFGA VSEYINVVSYS LCAMVIFTLTAYYQPLEGTP PYPYRTTVDY
601 IRLAGEVITLFTGVLEFFTN IKOLEMKKCP GVNSLFDGSGFQILYETYSV
651 LVIVSAALYL AGIEAYLAMV VFALVIGWMN ALYETRGLKL TGTYSIMK
701 ILFKDIERELVATHEMIGYASALVSLNLP CANMKVCNED QTNCVTPTYP
751 SCRDSSETFST FLDLFKLTI GMDLEMLSS TKYPVVEIIL LVITYILTSV
801 LILNMITALMGETVGQVSKE SKHIWKLQWA TTILDIERSF PVFLRKAERS
851 GEMVTVGKSS DGTPDRRWCF RVDEVNWSHW NQNLGIINED PGKNETYQYY
901 GFSHTVGRLR RDRWSSVPR VVELNKNSNP DEVVPLDSM GNPRCDGHQV
951 GYPRKWRTDD APL

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Key

 Transmembrane domains

 Ankyrin binding domains

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FIG. 21

A MULTIPLE COMPARISON OF THE AMINO ACID SEQUENCES OF THE RAT VR1 AND THE HUMAN VANILLOID RECEPTORS, hVR1, hVRL-1 AND hVR3

	10	20	30	40	50	(SEQ ID NO:3)
VR1	-----	-----	-----	-----	-----	
hVR1	-----	-----	-----	-----	-----	(SEQ ID NO:2)
hVRL-1	-----	-----	-----	-----	-----	(SEQ ID NO:6)
hVR3	MPRVVGP	GANLCFQV	RERGSCSS	RRLRLA	ANHIWEWPP	CAPVITTVALKQ (SEQ ID NO:5)
	60	70	80	90	100	
VR1	-----	-----	-----	-----	-----	
hVR1	-----	-----	-----	-----	-----	
hVRL-1	-----	-----	-----	-----	-----	
hVR3	LAALLLVH	VGGGFLEP	PPLAGFCL	TPLSFPC	RLLSSADG	PGAGMADSSEGP
	110	120	130	140	150	
VR1	-----	-----	-----	-----	-----	
hVR1	-----	-----	-----	-----	-----	
hVRL-1	-----	-----	-----	-----	-----	
hVR3	RAGPGEVA	ELPGDESG	TPGGEAF	PLSSLANL	FEGEDG	SLSPSPADASRPA
	160	170	180	190	200	
VR1	DPDRDPNCK	PPFVKPHI	FTTSTRTR	LEF...	KGDSEAS	PLDCPYEEG
hVR1	DPLDGPNSR	PPPAKPOL	STAKSRTR	LEF...	KGDSEEA	FPVDCPHEEG
hVRL-1	-----	-----	-----	-----	-----	
hVR3	GGGGRPNL	RMKFQGA	ERKGVNP	IDLLEST	LYESSVVP
	210	220	230	240	250	
VR1	GLASCPIT	VSSVLT	IQRPDGP	ASVREPS	SODSVSAG	EKP.PRLYDRRS
hVR1	ELDSCET	ITVSEV	ITIQRPD	GPTGAR	LLSODSVA	STEKT.LRLYDRRS
hVRL-1	GSGLPEM	..ESQFQ	GEDRKF	APQIRV	NLNYRK	GTGASQPD
hVR3	MDSLE	DYGT	YRHSSD	NKRW	KKIIEK	QPSKAPAP
	260	270	280	290	300	
VR1	IFDAVACS	NGOELE	SELPL	QSKGR	LTDESE	FKDPE
hVR1	IFDAVACS	NGOELE	SELPL	QSKGR	LTDESE	FKDPE
hVRL-1	LENAVSR	GVPE	DLAGL	PEYLSK	TSKYLT	DSEYTE
hVR3	LENAVSR	GVPE	DLAGL	PEYLSK	TSKYLT	DSEYTE
	310	320	330	340	350	
VR1	NGNDT	IAILL	DVAR	CTD	SLKQ	VNASY
hVR1	NGNDT	IAILL	DVAR	CTD	SLKQ	VNASY
hVRL-1	DGVNAC	ILPL	QIDR	SGNP	QPLV	NAQC
hVR3	NGNDT	IAILL	DVAR	CTD	SLKQ	VNASY
	360	370	380	390	400	
VR1	VTLVENG	ADVOA	AANGD	FFKKT	KGRP	GEYFGE
hVR1	VTLVENG	ADVOA	AANGD	FFKKT	KGRP	GEYFGE
hVRL-1	VTLVENG	ADVOA	AANGD	FFKKT	KGRP	GEYFGE
hVR3	VTLVENG	ADVOA	AANGD	FFKKT	KGRP	GEYFGE
	410	420	430	440	450	
VR1	LEONSWO	PADIS	ARDSV	GNIVL	HALV	ADNTVD
hVR1	LEONSWO	PADIS	ARDSV	GNIVL	HALV	ADNTVD
hVRL-1	LEENPHO	PASLO	ATDSQ	GNIVL	HALV	MTSDNSA
hVR3	LEENPHO	PASLO	ATDSQ	GNIVL	HALV	MTSDNSA
	460	470	480	490	500	
VR1	AKEDT	IKLEE	ITNR	GLT	PLALA	NSCKIG
hVR1	AKEDT	IKLEE	ITNR	GLT	PLALA	NSCKIG
hVRL-1	AKEDT	IKLEE	ITNR	GLT	PLALA	NSCKIG
hVR3	AKEDT	IKLEE	ITNR	GLT	PLALA	NSCKIG

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